Molecular Markers in Fine Needle Aspirates of the Thyroid

Policy Number: AHS – M2108 – Molecular Markers in Fine Needle Aspirates of the Thyroid
Prior Policy Name and Number, as applicable: 
Initial Presentation Date: 06/16/2021
Revision Date: N/A

I. Policy Description

Thyroid nodules are growths or swellings on the thyroid gland. These nodules may be caused by several different disorders such as thyroiditis or cysts. However, these nodules may also be caused by thyroid cancer, which occurs in 4-6.5% of nodules. A biopsy is often performed to assess the histological components of this nodule, usually through a fine needle aspiration or FNA. A 23 to 27 gauge (commonly 25 gauge) needle is used, with or without local anesthesia. This technique can obtain an acceptable sample in 90-97% of solid nodules (D Ross, 2019).

Molecular markers such as genetic mutations or miRNA expression may be used to help identify malignant nodules. Mutational analysis by sequencing or PCR can identify mutations such as \textit{BRAF}, \textit{RAS}, \textit{RET}/\textit{PTC}, and \textit{PAX8}/\textit{PPARy}. Another tool is a gene expression classifier, which measures mRNA to determine the activity level of a number of genes and uses an algorithm to predict malignancy based on gene expression (Nikiforov, Yip, & Nikiforova, 2013).

II. Related Policies

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<thead>
<tr>
<th>Policy Number</th>
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<tr>
<td>AHS-G2045</td>
<td>Thyroid Disease Testing</td>
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<td>AHS-M2066</td>
<td>Genetic Cancer Susceptibility Using Next Generation Sequencing</td>
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<td>AHS-M2078</td>
<td>Genetic Testing for Germline Mutations of the RET Proto-Oncogene</td>
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<td>AHS-M2087</td>
<td>Genetic Testing for PTEN Hamartoma Tumor Syndrome</td>
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<td>AHS-M2109</td>
<td>Molecular Panel Testing of Cancers for Diagnosis, Prognosis, and Identification of Targeted Therapy</td>
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<tr>
<td>AHS-G2124</td>
<td>Serum Tumor Markers for Malignancies</td>
</tr>
</tbody>
</table>
III. Indications and/or Limitations of Coverage

Application of coverage criteria is dependent upon an individual’s benefit coverage at the time of the request.

1. Mutation analysis (Eg. *BRAF* V600, *RET/PTC*, *RAS*, *PAX8/PPAR*) or the use of a gene expression classifier in fine-needle aspirates of the thyroid, in an adult individual (>18 years of age) being evaluated for thyroid carcinoma, that are cytologically characterized according to Bethesda criteria as atypia of undetermined significance (AUS) or follicular lesion of undetermined significance (FLUS) (Bethesda-III), follicular neoplasm (FN) or suspicious for follicular neoplasm (SFN) (Bethesda-IV) **IS MEDICALLY NECESSARY** to assist in patient management decisions. See Notes 1 and 2 for further guidance.

2. Mutation analysis (Eg. *BRAF* V600, *RET/PTC*, *RAS*, *PAX8/PPAR*) or the use of a gene expression classifier in fine-needle aspirates of the thyroid that are cytologically characterized according to Bethesda criteria as suspicious for malignancy (Bethesda-V) or malignant (Bethesda-VI) **IS NOT MEDICALLY NECESSARY** in adult patients (>18 years of age) being evaluated for thyroid carcinoma.

   *The following does not meet coverage criteria due to a lack of available published scientific literature confirming that the test(s) is/are required and beneficial for the diagnosis and treatment of a patient’s illness.*

3. Mutation analysis (Eg. *BRAF* V600, *RET/PTC*, *RAS*, *PAX8/PPAR*) or the use of a gene expression classifier in fine-needle aspirates of the thyroid **IS INVESTIGATIONAL** in pediatric patients (<18 years of age) being evaluated for thyroid carcinoma.

4. The microRNA profiling tests in fine-needle aspirates of the thyroid that are cytologically characterized as follicular or Hürthle cell neoplasm (FN) / suspicious for follicular neoplasm (SFN), atypia of undetermined significance (AUS) or follicular lesion of undetermined significance (FLUS), papillary or anaplastic neoplasms **IS INVESTIGATIONAL** in adult (>18 years of age) and pediatric (<18 years of age) patients being evaluated for thyroid carcinoma.

5. Mutation analysis (Eg. *BRAF* V600, *RET/PTC*, *RAS*, *PAX8/PPAR*) or the use of gene expression classifier or the microRNA profiling tests **IS INVESTIGATIONAL** in adult (>18 years of age) and pediatric (<18 years of age) patients in all other situations.

Note 1: Only tests up to 50 genes are allowed; testing more than 50 genes is strictly prohibited.

Note 2: The table below gives examples of commercially available tests and the number of genes (or RNA sequences) included within the test as of 03/11/2020. Inclusion of a test on this table does NOT indicate positive coverage.
<table>
<thead>
<tr>
<th>Test Name</th>
<th>Number of Genes/RNAs</th>
<th>Company</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>ThyGeNEXT</td>
<td>10 genes</td>
<td>Interpace Diagnostics</td>
<td>NGS panel of 10 genes (ALK, BRAF, GNAS, HRAS, K Ras, NRAS, PIK3CA, PTEN, RET, TERT)</td>
</tr>
<tr>
<td>ThyraMIR</td>
<td>10 miRNAs</td>
<td>Interpace Diagnostics</td>
<td>miRNA expression analysis, 10 miRNAs, intended to identify malignancy in ThyGeNEXT results of no or “weak” malignancy</td>
</tr>
<tr>
<td>NeoTYPE Thyroid profile</td>
<td>26 genes</td>
<td>NeoGenomics</td>
<td>Gene analysis “for evaluation of “fine needle aspirates of thyroid nodules that are indeterminate or suspicious on cytology”</td>
</tr>
<tr>
<td>ThyroSeq v3</td>
<td>112 genes</td>
<td>UPMC</td>
<td>Genomic classifier, intended to evaluate cytologically indeterminate nodules</td>
</tr>
<tr>
<td>Afirma GSC</td>
<td>167 genes</td>
<td>Afirma</td>
<td>Gene expression classifier, measures “activity level” of 167 genes</td>
</tr>
<tr>
<td>Afirma Xpression Atlas</td>
<td>511 genes</td>
<td>Afirma</td>
<td>Gene expression classifier, 511 genes, 761 variants, and 130 fusion pairs, intended to inform surgical and therapeutic decisions for high-risk patients</td>
</tr>
</tbody>
</table>

### IV. Scientific Background

FNA is a traditional diagnostic approach to differentiate malignant thyroid nodules that need surgery from benign nodules that do not require surgery. It offers definitive diagnosis in most cases; however, 20–30% of nodules yield an indeterminate cytologic diagnosis in which cancer cannot be ruled out. This may lead to suboptimal management of these patients and can result in unnecessary surgical interventions (Nikiforov et al., 2013). FNA results are normally categorized according to the National Cancer Institute into six categories. They are, in order of severity: nondiagnostic or unsatisfactory (Bethesda-I), benign (II), atypia of undetermined significance (AUS) or follicular lesion of undetermined significance (FLUS) (III), follicular neoplasm (FN) or suspicious for follicular neoplasm (SFN) (IV), suspicious for malignancy (V), and malignant, which includes lymphomas and carcinomas.
A benign result is reported in 60-70% of FNAs. Nondiagnostic does not refer to an absence of cancer; rather it means that the sample provided was inadequate for a conclusive result and another sample must be provided. Categories III and IV are typically referred to as “indeterminate” and typically have a malignancy risk of as high as 40%. Patients with indeterminate nodules will frequently have a surgery to treat the issue; however, up to 95% of these nodules are ultimately evaluated as benign. Further testing must be done with indeterminate cases to categorize these lesions (Cibas & Ali, 2009; Douglas Ross, 2018).

Molecular markers have been used to identify the true status of an indeterminate FNA. Assessing components of FNA aspirates such as micro-RNA, mutational status of certain genes, or genomic sequencing could prove useful, especially as these components can be reliably detected during the FNA itself (Hodak & Rosenthal, 2013; Xing, Haugen, & Schlumberger, 2013). Prior to the emergence of molecular markers as an indicator, a repeat FNA was often performed, ultimately leading to a surgery to remove the nodule. Molecular markers could thus reduce unnecessary surgeries as well as provide better risk stratification (Douglas Ross, 2018).

**Clinical Validity and Utility**

A study focusing on detection of $BRAF$, $RAS$, $RET/PTC$, and $PAX8/PPARY$ mutations found that detection of any mutation resulted in a risk of histologic malignancy of 88%, 87%, and 95% for samples of Bethesda categories III, IV, and V, respectively. 967 samples were taken, and 87 mutations were found. The risk of cancer in mutation-negative samples was 6%, 14%, and 28%, respectively. Unfortunately, there was also a 14% false negative rate, limiting the usefulness of this panel (Nikiforov et al., 2011; Douglas Ross, 2018).

Another study focusing on a next-generation sequencing (NGS) assay, additional point mutations (including $TERT$, $TP53$, and others), as well as gene fusions that occur in thyroid cancer was performed. 143 samples with a classification of follicular neoplasm or suspicious for follicular neoplasm were assessed, and the NGS panel “ThyroSeq v2” was used, which tests for point mutations in 13 genes and 42 types of gene fusions. 104 samples were found to be benign with the other 39 sample results being malignant. Overall, the NGS panel performed at a negative predictive value of 96%, a positive predictive value of 83%, 90% sensitivity, 93% specificity, and 92% accuracy for FN/SFN nodules (Nikiforov et al., 2014).

Another method that has been used is assessment of mRNA expression through a gene expression classifier. This is the basis of the Afirma genomic sequencing classifier, which identifies markers for features, such as medullary thyroid cancer, as well as distinguishes between Hürthle cell neoplasms from non-neoplastic Hürthle cell neoplasms. The second version of this test was assessed with 191 indeterminate samples. The sensitivity and specificity were 91% and 68%, respectively, and the negative and positive predictive values were 96% and 47%, respectively (Patel, Angell, Babiarz, & et al., 2018; Douglas Ross, 2018). Afirma has produced a similar gene expression classifier “Xpression Atlas” that can be used to assess B-III to B-VI category neoplasms. Xpression Atlas evaluates a total of 761 gene variants and 130 fusion pairs (Afirma, 2018; Douglas Ross, 2018).

The above methods may be combined to better evaluate neoplasms, for instance an miRNA gene expression classifier using both mutation analysis and miRNA expression. Labourier et al evaluated a diagnostic algorithm that combined mutation analysis and miRNA expression to assess preoperative
FNAs. This algorithm contained 17 validated gene alterations using the miRInform thyroid test and a 10-miRNA gene expression classifier. 109 samples of either AUS/FLUS or FN/SFN were evaluated with this combined algorithm, and this algorithm correctly identified 64% of malignant samples and 98% of benign ones. The sensitivity and specificity were 89% and 85%, respectively. For AUS/FLUS and FN/SFN, the negative predictive value was 97% and 91%, and the positive predictive value for malignancy was 68% and 82%, respectively (Labourier et al., 2015; Douglas Ross, 2018).

No single methodology has achieved clinical utility to reliably resolve all indeterminate cytology, and thus several professional organizations, including the American Association of Clinical Endocrinologists (AACE), the American Thyroid Association (ATA), and the National Comprehensive Cancer Network (NCCN), have published guidelines for the evaluation of thyroid nodules, all of which endorse a similar multistep strategy suggesting molecular markers can be of use when cytology is indeterminate, yet acknowledging its current limitations (Gharib et al., 2016; Haugen et al., 2016; NCCN, 2019).

Banizs et al evaluated the utility of “combined mutation analysis and microRNA classification in reclassifying cancer risk of cytologically indeterminate thyroid nodules”. A three-tiered microRNA threshold was determined based on nodules with known disease status, and an expected rate of malignancy calculated from mutation analysis and the microRNA approach was validated in an independent cohort of “atypia of undetermined significance or follicular lesion of undetermined significance (AUS/FLUS) and follicular neoplasm or suspicious for follicular neoplasm (FN/SFN) nodules with surgically derived outcomes”. From there, 2685 patients were included in the intended analysis. 82% of these samples lacked mutations, with BRAF, PIK3CA, PAX8/PPARγ, and RET/PTC mutations all comprising 2% or less. The maximum expected risk of malignancy in these nodules without mutation was 9% for AUS/FLUS nodules and 17% for FN/SFN nodules, but with positive microRNA status, these rates increased to 36% and 54% respectively. RAS mutations occurred in 15% of mutations, and the expected malignancy rates in nodules with RAS or PAX8/PPARγ mutations was 49% for AUS/FLUS nodules and 65% for FN/SFN nodules. With positive microRNA status, these rates increased to 85% and 91%, respectively (Banizs & Silverman, 2019).

Commercially Available Testing

Commercially available panels of molecular markers utilizing FNA specimens from the thyroid include the following tests:

ThyGeNEXT and ThyraMIR

ThyGeNEXT (Interpace Diagnostics, Parsippany, NJ) is a specific oncogene, mutational panel that tests genetic alterations across 10 genes associated with papillary carcinoma and follicular carcinoma. ThyGeNEXT uses next generation sequencing (NGS) platform to identify genetic alterations across those 10 genes, which are as follows: ALK, BRAF, GNAS, HRAS, KRAS, NRAS, PIK3CA, PTEN, RET, TERT. This test is primarily for category III or IV nodules. Recently, Interpace Diagnostics has developed a new molecular test, ThyraMIR. This test is based on microRNA analysis of the expression of 10 microRNAs. The manufacturer claims that this test can identify malignancy in nodules that indicate no or “weak” mutation for ThyGeNEXT. According to Interpace Diagnostics, combined test performance has negative predictive value of 94%, positive predictive value of 74%, with 89% sensitivity and 85%
specificity. The sensitivity and specificity for the ThyGeNEXT panel alone is 63% and 84%, respectively, for cases of indeterminant cytology (Interpace, 2019).

ThyroSeq v3

ThyroSeq is a test intended for assessment of thyroid nodules with undetermined cytology initially designed to target 12 cancer genes with 284 mutational hotspots. The latest version of this test, ThyroSeq v3, is based on NGS of DNA and RNA. This test detects 4 types of alterations; mutations, gene fusions, expression alterations, and copy number alterations. This test analyzes 112 genes, providing information on more than 12,000 mutation hotspots and more than 120 gene fusion types. First, the sample is ensured to have enough material to proceed (such as amount of thyroid follicular cells). Then, the NGS is performed and reviewed by a pathologist. Finally, the report is sent to the patient in about 2 weeks (UPMC, 2017).

In a multicenter study, this test was produced a negative predictive value of 97%, positive predictive value of 66%, with 94% sensitivity and 82% specificity in 257 cases of Bethesda-III and -IV nodules. The authors concluded that up to 61% of patients with indeterminate cytology could avoid diagnostic surgery through multigene genomic classifier testing (Steward, Carty, Sippel, & et al., 2018). Other NGS-based molecular tests for thyroid nodules are available from other labs.

Nikiforova et al evaluated the analytical performance of ThyroSeq v3 (intended to analyze 112 genes for alterations). A genomic classifier (GC) is used to differentiate malignant nodules from benign. 238 tissue samples and 175 FNA samples were included in the cohort. ThyroSeq detected over 100 genetic alterations. The GC cutoff identified malignant nodules in the tissue samples from benign at 93.9% sensitivity, 89.4% specificity, and 92.1% accuracy. For the FNA sample, the GC sensitivity was 98.0%, the specificity was 81.8%, and the accuracy was 90.9% (Nikiforova et al., 2018).

Afirma Series

The Afirma Genomic Sequencing Classifier (GSC) is offered by Afirma. This test is intended to assess indeterminate nodules (Bethesda categories III and IV) and “conclusively rule out” surgery (Afirma, 2020). This test is based on the Afirma Gene Expression Classifier (GEC), which measured the activity level of 167 genes. The GSC added several features to better classify nodules, such as classifiers for medullary thyroid cancer, parathyroid lesions, BRAF V600E mutations, and overall better discrimination of Hurthle cell neoplasms (Douglas Ross, 2018). Wei et al note that the GSC is an “updated” version of the GEC, available since 2011. Wei et al also published a study discussing proposed advantages of the GSC over the GEC, namely its improved specificity (as a weakness of the GEC was its inability to identify true oncocytic lesions in the “suspicious” category). The authors compared the results of indeterminate FNA specimens (Bethesda categories III and IV) that were tested by Afirma GEC or GSC. 272 tests (194 GEC, 78 GSC) were evaluated. 221 samples were classified as AUS/FLUS and 51 were classified as FN/SFN. Out of the 194 samples tested with GEC, 88 were considered benign (45.4%) whereas 52 of the 78 GSC samples were considered benign (66.7%). In the AUS/FLUS category, 47.1% of cases were considered benign by GEC whereas 71.2% were considered benign by GSC (Wei, Veloski, Sharda, & Ehya, 2019).

Another test is the Afirma Xpression Atlas (XA). This test is a comprehensive panel encompassing 511 genes, 761 variants, and 130 fusion pairs. Genes such as BRAF, RET, and the RAS pathway of genes are included in this panel. This test is intended to inform surgical and therapeutic decisions for high-risk
patients. Afirma lists three populations for this test; those who are “suspicious” per the Afirma GSC test, Bethesda category V, and Bethesda category VI. Angell et al validated this test. The XA was compared to the results of multiple other methodologies (such as whole-transcriptome RNA-seq, targeted DNA-seq, et al) and concordance was evaluated. 943 blinded FNA samples were used to compare the DNA and RNA detection and 695 blinded FNAs were used to compare the fusion detections. At the cutoff of 5% variant frequency, 74% of allele variants detected by traditional methods were also detected by the XA, with 88% detected by the XA at a 20% variant frequency cutoff. 82% of fusions detected by the targeted RNA fusion assay were detected by the XA. From an analytical validity standpoint, intra-plate reproducibility was found to be 89%-94%, and inter-plate reproducibility was found to be 86%-91% (Angell et al., 2019).

Jug et al evaluated the performances of ThyroSeq and Afirma GEC within the context of ultrasonographic features and with the incorporation of the “noninvasive follicular thyroid neoplasm with papillary-like nuclear features (NIFTP) nomenclature”. The ultrasonographic pattern evaluations were derived from the 2015 American Thyroid Association guidelines. A total of 304 cases were evaluated, with 119 resections. All cases that met criteria for NIFTP were considered high-risk by both tests. However, when these NIFTP cases were moved from the malignant to the non-malignant category, the positive predictive value of ThyroSeq dropped from 42.9% to 14.3% and Afirma’s dropped from 30.1% to 25.3% (Jug, Datto, & Ji, 2018).

Endo et al compared Afirma’s GEC to its Gene Sequencing Classifier (GSC), using cytologically indeterminate nodules. 343 GEC-tested nodules and 164 GSC-tested nodules were identified. The GSC was found to have a “statistically significant higher benign call rate (76.2% vs. 48.1%), PPV (60.0% vs. 33.3%), and specificity (94.3% vs. 61.4%)”. The authors noted that the improvement was statistically significant for Bethesda III and IV nodules. The GSC benign call rate was significantly higher in Hürthle cell changes (88.8% vs 25.7%). 52.5% of indeterminate nodules went to surgery when using the GEC compared to only 17.6% when using the GSC (Endo et al., 2019).

RosettaGX Reveal

The RosettaGX Reveal test from Rosetta Genomics is a micro-RNA-based diagnostic test that evaluates indeterminate thyroid nodules. The test measures 24 sequences of miRNA through quantitative RTPCR, as well as a medullary carcinoma marker (hsa-miR-375). Each sample is classified as benign or suspicious for malignancy.

The test has a claimed negative predictive value of 99%, a 98% sensitivity, and a 78% specificity from a sample of 150 in which three pathologists all agreed on the evaluation. Out of the 189 total samples, the negative predictive value was 91%, sensitivity was 85%, and specificity was 72%. The nodule sizes were >0.5 cm. The authors concluded that this test may be able to differentiate between malignant and benign from a previously evaluated indeterminate sample (Lithwick-Yanai et al., 2017). This test has been discontinued.

NeoGenomics

NeoGenomics offers a “NeoTYPE” thyroid profile, intended for evaluation of “fine needle aspirates of thyroid nodules that are indeterminate or suspicious on cytology”. NeoGenomics states that FISH detects mutations and other gene rearrangements, and that BRAF mutation V600E is associated with
poor prognosis of papillary thyroid carcinoma. The test measures the following genetic features: “AKT1, ALK, ARID1A, ATM, BRAF, CDKN2A, CTNNB1, ERBB2, ERBB4, HRAS, KRAS, MEN1, MET, Microsatellite Instability (MSI), NF1, NF2, NRAS, PIK3CA, PTEN, RET, SMAD4, SMO, SRC, TERT Promoter, TP53, TSC1, TSC2, Tumor Mutation Burden (TMB), MET and RET by FISH, PD-L1, and PanTRK (NeoGenomics, 2020).

V. Guidelines and Recommendations

National Comprehensive Cancer Network (NCCN, 2019)

NCCN guidelines for thyroid carcinoma state that molecular diagnostic testing to detect individual mutations (such as BRAF V600E or RET/PTC) or pattern recognition approaches using molecular classifiers may be useful in the evaluating indeterminate FNA samples to assist in management decisions. NCCN states molecular diagnostics may be used to reclassify indeterminate lesions such as AUS/FLUS. Molecular markers should be interpreted within the context of each patient. The NCCN states that molecular testing may be considered to drive treatment decisions, and some mutations may have prognostic importance.

The NCCN further clarifies that, “The choice of the precise molecular test depends on the cytology and the clinical question being asked. Indeterminate groups include: 1) follicular or Hürthle cell neoplasms; and 2) AUS/FLUS.” The NCCN panel recommends molecular diagnostic testing for evaluating FNA results that are suspicious for follicular cell neoplasms or AUS/FLUS (category 2A) and does not recommend testing on suspected Hürthle cell neoplasms as studies historically do not perform well on Hürthle neoplasms. However, the NCCN has acknowledged some promising studies for assessing Hürthle neoplasms, with both the Afirma and ThyroSeq v3 tests providing better evaluations than their predecessors. The NCCN notes that molecular diagnostic testing may include individual mutation analysis or a multigene assay such as a gene expression classifier. Molecular markers may also help with treatment decisions or with eligibility in clinical trials.

The NCCN further notes that active surveillance may be an option for patients whose molecular diagnostics demonstrate a risk of malignancy under 5% and that the predictive value of molecular diagnostics may be influenced by pre-test probability of disease based on various FNA cytology groups. The NCCN states that molecular diagnostic testing may be useful for follicular cell carcinomas and diagnosing NIFTP, although current tests for assessing NIFTP have not been validated. Molecular testing is not recommended for diagnosing anaplastic thyroid carcinoma. Finally, the NCCN highlights that the diagnostic utility of molecular diagnostics in pediatric patients is still unclear because most of the published literature is on adult patients with thyroid nodules (NCCN, 2019).

American Thyroid Association (ATA, 2015)

The 2015 ATA guidelines on the management of adult patients with thyroid nodules and differentiated thyroid cancer make the following recommendations on the use of molecular markers:

• “If molecular testing is being considered, patients should be counseled regarding the potential benefits and limitations of testing and about the possible uncertainties in
the therapeutic and long-term clinical implications of results”. (Strong recommendation; low-quality evidence)

• “If intended for clinical use, molecular testing should be performed in Clinical Laboratory Improvement Amendments/College of American Pathologists (CLIA/CAP) certified molecular laboratories, or the international equivalent, because reported quality assurance practices may be superior compared to other settings”. (Strong recommendation; low-quality evidence)

• “For nodules with AUS/FLUS cytology, after consideration of worrisome clinical and sonographic features, investigations such as repeat FNA or molecular testing may be used to supplement malignancy risk assessment in lieu of proceeding directly with a strategy of either surveillance or diagnostic surgery. Informed patient preference and feasibility should be considered in clinical decision-making”. (Weak recommendation; moderate-quality evidence)

• “Diagnostic surgical excision is the long-established standard of care for the management of FN/SFN cytology nodules. However, after consideration of clinical and sonographic features, molecular testing may be used to supplement malignancy risk assessment data in lieu of proceeding directly with surgery. Informed patient preference and feasibility should be considered in clinical decision-making”. (Weak recommendation; moderate-quality evidence)

• In general, only nodules >1 cm should be evaluated as they have a greater chance to become a clinically significant cancer. However, there are some cases where nodules <1 cm may be evaluated due to other clinical symptoms. The ATA states that “attempts to diagnose and treat all such small thyroid cancers in an effort to prevent exceedingly rare outcomes is deemed to cause more harm than good”.

• “If the nodule is benign on cytology, further immediate diagnostic studies or treatment are not required”.

• “Each nodule that is >1 cm carries an independent risk of malignancy and therefore multiple nodules may require FNA”.

The guidelines also state that “there is currently no single optimal molecular test that can definitively rule in or rule out malignancy in all cases of indeterminate cytology, and long-term outcome data proving clinical utility are needed” (Haugen et al., 2016).

The ATA Guidelines Task Force on Pediatric Thyroid Cancer have developed unique guidelines for children and adolescents with thyroid tumors. They have presented 34 recommendations including recommendations on molecular markers testing and nodules. The ATA panel recommended the pediatric age to be limited to a patient that is < 18 years of age to more accurately define the impact of the physiologic changes of growth and development on tumor behavior. These guidelines note that a size criterion is more difficult in children as age affects volume greatly and size of the nodule is not
predictive of malignancy. Overall, studies focusing on molecular diagnostics in children have not been validated and so cannot be recommended at this time (Francis et al., 2015).

**American Academy of Pediatrics (AAP, 2018)**

The AAP endorsed the guidelines of the American Thyroid Association Guidelines Task Force on Pediatric Thyroid Cancer (as presented in (Francis et al., 2015)) in a publication released in 2018 (AAP, 2018).

**American Association of Clinical Endocrinologists (AACE), American College of Endocrinology (ACE) and Associazione Medici Endocrinologi (AME, 2016)**

These joint guidelines recommend the following:

- “Molecular testing should be considered to complement, not replace cytologic evaluation, and only if the results are expected to influence clinical management. As a general rule, molecular testing is not recommended in nodules with established benign or malignant cytologic characteristics.”

- FNA is recommended for high ultrasound (US) risk lesions of ≥10 mm, intermediate risk lesions of >20 mm, and low risk lesions >20 mm and growing or with a risk history.
  - FNA is not recommended for nodules that are functional on scintigraphy.

- Repeat FNA is recommended in benign nodules with suspicious clinical or US findings, a nondiagnostic initial FNA on a solid nodule, and nodules that become symptomatic or increase in volume by 50%. Pregnancy does not affect cytologic diagnostic criteria. Routine repeat FNA is generally not necessary.

- Nodules with a major diameter of <5 mm should be monitored instead of biopsied.

- Consider the detection of BRAF and RET/PTC and possibly PAX8/PPARG and RAS mutations if available.

- There is no stance on gene expression classifiers for indeterminate nodules, due to insufficient evidence and limited follow-up.

- There is also insufficient evidence to take any stance on mutation testing to guide surgery decisions, except on mutations with a PPV approaching 100% for papillary thyroid carcinoma such as BRAFV600E (Gharib et al., 2016).

**European Thyroid Association (ETA, 2017)**

The ETA states that molecular testing for BRAF, RET/PTC and possibly PAX8, PPARG, and RAS mutations may be considered for cytologically indeterminate lesions. The search for molecular markers in B-II class lesions is not recommended, although one member of the panel did not agree with this non-recommendation. A GEC test cannot be recommended to exclude malignancy to replace diagnostic
surgery or close surveillance, although one member did not agree with this nonrecommendation. The targeted NGS approach is considered the most promising, with larger panels potentially becoming a rule-in and rule-out test if >95% negative predictive value can be reached. The ETA notes that molecular diagnostics may reduce completion thyroidectomies or other surgeries due to clearer assessments of an indeterminate lesion (Paschke et al., 2017).

**European Society for Medical Oncology (ESMO, 2019)**

ESMO remarks that preoperative FNA for cytology is “not required” for nodules 1 cm or smaller. ESMO states that FNA diagnosis “can be facilitated” by assessment of malignancy markers and molecular alterations. Specifically designed gene panels are “reportedly” useful for identifying malignancy with indeterminate samples (Filetti et al., 2019).

**VI. State and Federal Regulations, as applicable**

Commerciaally available panels of molecular markers utilizing FNA specimens from the thyroid are currently considered to be laboratory-developed tests (LDTs), which are regulated by the Centers for Medicare and Medicaid (CMS) as high-complexity tests under the Clinical Laboratory Improvement Amendments of 1988 (CLIA ’88). As an LDT, the U.S. Food and Drug Administration has not approved or cleared this test; however, FDA clearance or approval is not currently required for clinical use.

**VII. Applicable CPT/HCPCS Procedure Codes**

<table>
<thead>
<tr>
<th>Code Number</th>
<th>Code Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>81210</td>
<td>BRAF (B-Raf proto-oncogene, serine/threonine kinase) (eg, colon cancer, melanoma), gene analysis, V600 variant(s)</td>
</tr>
<tr>
<td>81401</td>
<td>Molecular pathology procedure, Level 2</td>
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<tr>
<td></td>
<td>Genes:</td>
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<tr>
<td></td>
<td><strong>PAX8/PPARG</strong> (t(2;3) (q13;p25)) (eg, follicular thyroid carcinoma), translocation analysis</td>
</tr>
<tr>
<td>Code</td>
<td>Code Description</td>
</tr>
<tr>
<td>-------</td>
<td>-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------</td>
</tr>
<tr>
<td>81445</td>
<td>Targeted genomic sequence analysis panel, solid organ neoplasm, DNA analysis, and RNA analysis when performed, 5-50 genes (eg, ALK, BRAF, CDKN2A, EGFR, ERBB2, KIT, KRAS, NRAS, MET, PDGFRA, PDGFRB, PGR, PIK3CA, PTEN, RET), interrogation for sequence variants and copy number variants or rearrangements, if performed</td>
</tr>
<tr>
<td>81455</td>
<td>Targeted genomic sequence analysis panel, solid organ or hematolymphoid neoplasm, DNA analysis, and RNA analysis when performed, 51 or greater genes (eg, ALK, BRAF, CDKN2A, CEBPA, DNMT3A, EGFR, ERBB2, EZH2, FLT3, IDH1, IDH2, JAK2, KIT, KRAS,</td>
</tr>
<tr>
<td>81479</td>
<td>Unlisted molecular pathology procedure</td>
</tr>
<tr>
<td>81546</td>
<td>Oncology (thyroid), mRNA, gene expression analysis of 10,196 genes, utilizing fine needle aspirate, algorithm reported as a categorical result (eg, benign or suspicious) Proprietary test: Afirma® Genomic SequencingClassifier Lab/Manufacturer: Veracyte, Inc</td>
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<tr>
<td>81599</td>
<td>Unlisted multianalyte assay with algorithmic analysis</td>
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<tr>
<td>Test Code</td>
<td>Test Description</td>
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| 0018U     | Oncology (thyroid), microRNA profiling by RT-PCR of 10 microRNA sequences, utilizing fine needle aspirate, algorithm reported as a positive or negative result for moderate to high risk of malignancy.  
**Proprietary Test:** ThyraMIR® |
| 0026U     | Oncology (thyroid), DNA and mRNA of 112 genes, next-generation sequencing, fine needle aspirate of thyroid nodule, algorithmic analysis reported as a categorical result (“Positive, high probability of malignancy” or “Negative, low probability of malignancy”).  
**Proprietary Test:** ThyroSeq® |

**VIII. Evidence-based Scientific References**


AND ASSOCIAZIONE MEDICI ENDOCRINOLOGI MEDICAL GUIDELINES FOR CLINICAL PRACTICE
FOR THE DIAGNOSIS AND MANAGEMENT OF THYROID NODULES – 2016 UPDATE.
http://dx.doi.org/10.4158/EP161208.GL. doi:i1530-891X-22-s1-1


Ross, D. (2019). Diagnostic approach to and treatment of thyroid nodules - UpToDate. In D. Cooper (Ed.), *UpToDate*. Waltham. MA.


### IX. Revision History

<table>
<thead>
<tr>
<th>Revision Date</th>
<th>Summary of Changes</th>
</tr>
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<tbody>
<tr>
<td>06/16/2021</td>
<td>Initial presentation</td>
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